MRID No. 422563-05

DATA EVALUATION RECORD 129099

1. **CHEMICAL:** NTN 33893.
Shaughnessey No. ~~129059.~~

2. **TEST MATERIAL:** NTN 33893 technical; Batch No. 1717119/89: 96.2% active ingredient; and Batch No. 17129-90: 95.8% active ingredient; a yellow-colored powder.

3. **STUDY TYPE:** 72-3. Mollusc 96-Hour Shell Deposition Study. Species Tested: Eastern Oyster (*Crassostrea virginica*).

4. **CITATION:** Wheat, J. and G.S. Ward. 1991. NTN 33893 Technical: Acute Effect on New Shell Growth of the Eastern Oyster, *Crassostrea virginica*. Report No. 101978. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 422563-05.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 9/28/92 Concurred 11/25/92 EFED/EEB

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 9/28/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: Concurred
Date: 12/14/92

7. **CONCLUSIONS:** The first study is not scientifically sound because the control oyster growth was less than the minimum requirement (2 mm). The second study is scientifically sound and meets the guideline requirements for a mollusc shell deposition study. Based on the results of the second study, the 96-hour EC_{50} was >145 mg a.i./l (mean measured concentration) which classifies NTN 33893 as practically non-toxic to eastern oysters. The NOEC could not be determined.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

2049919

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Eastern oysters (*Crassostrea virginica*) were obtained from a commercial supplier in Dennis, MA. The oysters were held in the laboratory, in natural unfiltered seawater, for 2-6 days prior to testing. At the initiation of the holding period, 2-5 mm of shell margin was ground from each oyster with a grinding wheel to provide a smooth flattened edge. The salinity of the seawater ranged from 30 to 36 parts per thousand (ppt) and the temperature was 19.9-24.4°C.

The dilution water control oysters used in the first test had an average length (umbo to distal valve edge) of 21.5 (19.2-23.7) mm and an average wet weight of 0.31 (0.21-0.41) g. The control oysters used in the second test had an average length of 24.3 (19.5-28.0) mm and an average wet weight of 0.52 (0.35-0.86) g.

B. Test System: The test system for the two tests were different. "In the first test, the exposure system consisted of a glass head box fitted with glass tubing calibrated to provide unfiltered saltwater to each test chamber at a rate of approximately 400 ml/minute. This flow rate was sufficient to provide a minimum of approximately 1.2 l of dilution water per oyster per hour." The primary toxicant stock solution (384,800 mg a.i./l) was prepared in dimethylformamide (DMF). The solution was stirred overnight, allowed to settle for 1 day, then filtered. The filtrate concentration was 276,500 mg a.i./l. Four additional stock solutions were prepared by serial dilution. The stock solutions were continuously delivered to glass mixing boxes, where the test solutions were prepared. The test chambers were 29-l glass aquaria designed to maintain a solution height of 13 cm and a test volume of 19 l. The flow rate provided 30 volume additions/container/day.

The second test was performed using a glass head box fitted with glass tubing calibrated to provide a flow of dilution water of 365 ml/min. The flow of toxicant stock solution was approximately 135 ml/min giving a total flow rate of 500 ml/min (approximately 1.0 l/oyster/hour). The test containers were 11.3-l glass aquaria containing 5.4 l of solution at a depth of 6 cm. The flow rate provided 133 volume additions/container/day. The stock solution (500 mg

a.i./l) for this test was prepared by mixing 104.4 g of NTN 33893 (Batch No. 17129-90) with 750 ml of seawater in a high speed blender. The mixture was diluted with 199.25 l of unfiltered seawater and stirred overnight.

All test chambers were randomly positioned in a water bath under a 16-hour light/8-hour dark photoperiod with 15-minute dawn and dusk simulations. Light intensity during the test was 304 to 508 lux.

Natural unfiltered seawater with a salinity of 30-35 ppt was used as test dilution water.

C. **Dosage:** Ninety-six-hour flow-through tests. Based on the results of a preliminary test, the first definitive test consisted of five nominal concentrations (2.6, 4.3, 7.2, 12.0, and 19.4 mg a.i./l), a dilution water control, and a solvent control (70 µl/l DMF). The second definitive test consisted of a single concentration (121.5 mg a.i./l) and a dilution water control.

D. **Design:** Just prior to test initiation, oysters which demonstrated shell growth during holding were carefully ground to remove all new shell growth. In the first test, the prepared oysters were impartially added, two at a time, to the test chambers for a total of 20 per concentration. In the second test, 30 oysters were used per concentration. One chamber was used per treatment in both tests. No supplemental food was added.

Observations of mortality and test solutions were made every 24 hours. At the end of the test, oyster growth was measured to the nearest 0.1 mm. The dissolved oxygen concentration (DO) and pH of the test solutions were measured in each chamber at the beginning of the test and at each 24-hour observation. The salinity of the dilution water control was measured daily. The temperature was monitored hourly in the control chamber using a data logging device.

The test concentrations were measured using high pressure liquid chromatography fitted with an ultra-violet detector. During test 1, the solutions were measured at test initiation and termination. During test 2, the solutions were measured daily.

E. **Statistics:** Dilution water control and solvent control growth were compared using a t-test. Exposed oyster

responses were compared to the pooled control using analysis of variance (ANOVA) and Dunnett's test. In the second test, the growth of exposed oysters were compared to that of the dilution water control using a t-test.

12. **REPORTED RESULTS:** The test systems functioned properly during the exposures. During the first test, the mean measured concentrations were 2.93, 5.14, 8.19, 14.2, and 23.3 mg a.i./l (Table 1, attached). These values ranged from 113 to 120% of nominal concentrations. Undissolved test material was observed in the two highest exposure levels throughout the exposure period. One observation of undissolved material was made in the 8.19 mg a.i./l concentration. In the single exposure test, the mean measured concentration was 145 mg a.i./l which was 119% of nominal concentration (Table 8, attached).

Mean new shell growth for the dilution water control and solvent control during the first test was 1.52 and 1.76 mm, respectively (Table 3, attached), and were not significantly different. Exposure to concentrations up to 23.3 mg a.i./l had no effect on new shell deposition, therefore the 96-hour EC_{50} for the first test was >23.3 mg a.i./l. The no-observed-effect concentration (NOEC) was 23.3 mg a.i./l.

In the single concentration test using 145 mg a.i./l, new shell growth was reduced by 22% compared to the dilution water control (Table 10, attached). This difference was statistically significant using the t-test. Mean new shell growth in the dilution water control was 2.89 mm. The 96-hour EC_{50} was >145 mg a.i./l and the NOEC could not be calculated. There was no mortality during either test.

Dissolved oxygen concentrations were at least 70% of saturation during both tests. The salinity during the first test was 32-35 ppt and 30 ppt during the second test. The pH values ranged from 7.6 to 8.1. The temperature during the first test was 20.1-22.5°C and 21.7-25.4°C during the second test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author presented no conclusions.

A Good laboratory practice statement was included in the report, indicating that the study was conducted in accordance with Good Laboratory Practice Standards set forth in 40 CFR Part 160. The dates and types of quality assurance audits were also included.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with the SEP, except for the following:

An amendment to the SEP states that control oysters must deposit a minimum of 2 mm of new shell in 96 hours. At the end of the first test, the control and solvent control oysters deposited an average of 1.52 and 1.76 mm.

In this study, the flow rate of the test solution was about 1.0-1.2 l/oyster/hour. According to the protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976), each oyster should receive a minimum of 5 L of flow-through test solution per hour.

As the authors stated, the oysters were held in the laboratory for less than the required 10 days.

The oysters should be arranged in the test aquaria with the cupped-valve down and the anterior hinged ends oriented in one direction. The authors did not describe the positioning of the oysters.

B. Statistical Analysis: The raw new shell deposition data from both tests were analyzed to determine the NOEC. The data from the first test did not meet the assumptions of normality and homogeneity of variances. The data were analyzed using the Kruskal-Wallis test. Average growth for several exposure groups were significantly higher than dilution water control and solvent control oysters (see attached printout 1). The NOEC for this test was 23.3 mg a.i./l. Growth inhibition >50% was not observed in this test, therefore EC_{50} calculations were not possible.

The data from the second test were analyzed using Student's t-test. Mean new shell growth in the exposure group was significantly lower than the control growth (see attached printout 1) therefore an NOEC could not be determined in this test. As above, an EC_{50} calculation was not possible.

C. Discussion/Results: Average new shell growth in control oysters (1.52 and 1.76 mm) at the conclusion of test 1 was lower than required (2.0 mm) in an amendment to the SEP. However, average growth in the control oysters during the second test was 2.89 mm. The test material could be considered practically non-toxic

Table 1. NTN-33893 Technical: Measured Concentrations During a 96-Hour Exposure of Eastern Oysters, Crassostrea virginica, Under Flow-Through Conditions

Nominal Concentration (mg/L; ppm)	Measured Concentration (mg/L) 0 Hr	96 Hr	Mean (±SD)		Percent of Nominal
Control	ND	ND	ND		---
Sol. Control	ND	ND	ND		---
2.6	2.72	3.14	2.93	(0.30)	113
4.3	4.88	5.40	5.14	(0.37)	120
7.2	7.79	8.59	8.19	(0.56)	114
12	13.6	14.8	14.2	(0.85)	118
19.4	17.7	28.9	23.3	(7.9)	120
SPIKE RECOVERY DATA					
MS (Rep A)	10.6	9.92	10.4	(0.43)	104
(Rep B)	10.2	10.9			

SD = Standard Deviation.

ND = Not detected; the limit of detection for the method was 0.005 mg/L.

MS = Matrix spike. The matrix spike consisted of test substance in dilution water. The spike concentration was 10 mg/L and conducted in duplicate.

Table 3. NTN-33893 Technical: New Shell Growth of the Eastern Oyster (Crassostrea virginica) Exposed for 96 Hours Under Continuous Flow-Through Conditions

Mean Measured Concentration (mg/L; ppm)	New Shell Growth (in mm) Treatment Mean (SD)	Difference from Control[a]	Percent[b] Change
Control	1.52 (0.40)	----	---
Solvent Control	1.76 (0.64)	----	---
Pooled Controls	1.64 (0.54)	----	---
2.93	1.72 (0.64)	0.08	+ 5
5.14	1.94 (0.50)	0.30	+18
8.19	2.54 (0.68)	0.90	+55[c]
14.2	2.17 (0.85)	0.53	+32[c]
23.3	2.11 (0.83)	0.47	+29[c]

[a]Difference from pooled controls.

$$\text{[b]Percentage Change} = \frac{\text{Shell deposition of exposed oysters minus shell deposition of pooled control oysters}}{\text{Shell deposition of pooled control oysters}} \times 100$$

[c]Mean new shell growth is significantly greater than that of the pooled controls at P = 0.05.

Table 8. NTN-33893 Technical: Measured Concentrations During a 96-Hour Exposure of Eastern Oysters, Crassostrea virginica, Under Flow-Through Conditions

Nominal Conc. (mg/L; ppm)	Measured Concentration (mg/L) 0 Hr	24 Hr	48 Hr	72 Hr	96 Hr	Mean (±SD)	Percent of Nominal
Control	ND	ND	ND	ND	ND	ND	---
121.5	151	146	138	146	144	145 (4.7)	119
500.0[a]	509	514	567	549	514	531 (25.9)	106
SPIKE RECOVERY DATA							
MS (Rep A)					127	128 (0.7)	105
(Rep B)					128		

SD = Standard Deviation.

ND = Not detected; the limit of detection for the method was 1.0 mg/L.

MS = Matrix spike. The matrix spike consisted of test substance in dilution water. The spike concentration was 121.5 mg/L and conducted in duplicate.

[a] Values for stocks that were prepared daily.

Table 10. NTN-33893 Technical: New Shell Growth of the Eastern Oyster (Crassostrea virginica) Exposed for 96 Hours Under Continuous Flow-Through Conditions

Mean Measured Concentration (mg/L; ppm)	New Shell Growth (in mm) Treatment Mean (SD)	Difference from Control	Percent[a] Change
Control	2.89 (0.78)	----	----
145	2.24 (0.96)	0.65	-22[b]

[a]Percentage Change = (Shell deposition of exposed oysters minus Shell deposition of control oysters) / Shell deposition of control oysters X 100

[b]Mean new shell growth is significantly less than that of the control at P = 0.05.

422563-05, NTN 33893 technical, new shell deposition
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t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1.7550	CALCULATED t VALUE =	1.4274
GRP2 (BLANK CRTL) MEAN =	1.5200	DEGREES OF FREEDOM =	38
DIFFERENCE IN MEANS =	0.2350		

TABLE t VALUE (0.05 (2),40) = 2.021 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),40) = 2.704 NO significant difference at alpha=0.01

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	solvent control	1.755	1.755	1104.000
2	dilution contrl	1.520	1.520	802.000
3	2.93	1.715	1.715	1078.000
4	5.14	1.940	1.940	1435.000
5	8.19	2.540	2.540	2085.500
6	14.2	2.170	2.170	1727.500
7	23.3	2.110	2.110	1638.000

Calculated H Value = 36.089 Critical H Value Table = 12.590
Since Calc H > Crit H REJECT Ho:All groups are equal.

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 2 3 1 4 7 6 5
2	dilution contrl	1.520	1.520	\
3	2.93	1.715	1.715	. \
1	solvent control	1.755	1.755	. . \
4	5.14	1.940	1.940	. . . \
7	23.3	2.110	2.110	* . . . \
6	14.2	2.170	2.170	* . . . . \
5	8.19	2.540	2.540	* * * . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 12.804

Test 2 Statistical Evaluation - descriptive statistics

	N	MEAN	MEDIAN	TRMEAN	STDEV	SEMEAN
control	30	2.890	2.750	2.873	0.775	0.142
145 mg/l	30	2.237	2.200	2.258	0.959	0.175

	MIN	MAX	Q1	Q3
control	1.000	4.900	2.475	3.525
145 mg/l	0.000	4.000	1.575	3.000

TWOSAMPLE T FOR control VS 145 mg/l

	N	MEAN	STDEV	SE MEAN
control	30	2.890	0.775	0.14
145 mg/l	30	2.237	0.959	0.18

95 PCT CI FOR MU control - MU 145 mg/l: (0.20, 1.10)
TTEST MU control = MU 145 mg/l (VS NE): T= 2.90 P=0.0053 DF= 55

Mann-Whitney Confidence Interval and Test
control N = 30 Median = 2.7500
145 mg/l N = 30 Median = 2.2000
Point estimate for ETA1-ETA2 is 0.6000
95.2 pct c.i. for ETA1-ETA2 is (0.1999,1.1000)
W = 1090.0
Test of ETA1 = ETA2 vs. ETA1 n.e. ETA2 is significant at 0.0099
The test is significant at 0.0098 (adjusted for ties)

Test 2 RAW DATA

Printout # 2

ROW	control	145 mg/l
1	3.0	3.6
2	2.7	3.1
3	2.5	1.1
4	3.7	1.0
5	2.7	2.1
6	3.6	1.5
7	3.6	2.2
8	2.9	2.8
9	4.9	3.3
10	2.6	2.6
11	1.0	2.0
12	2.9	2.2
13	2.6	1.7
14	3.1	3.6
15	2.4	2.4
16	3.8	3.0
17	3.5	4.0
18	2.0	1.6
19	2.7	2.6
20	4.2	2.2
21	2.5	1.5
22	2.1	1.7
23	2.6	2.3
24	3.7	1.8
25	2.0	0.0
26	2.8	0.8
27	2.4	3.5
28	3.0	0.9
29	3.3	3.0
30	1.9	3.0